


**PATENT COOPERATION TREATY**

**PCT**

**INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY**

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference LP2015PC00	<b>FOR FURTHER ACTION</b>		See Form PCT/PEA/416
International application No. PCT/EP2004/012065	International filing date (day/month/year) 26.10.2004	Priority date (day/month/year) 27.10.2003	
International Patent Classification (IPC) or national classification and IPC C12N11/04, C12N11/08, C12P13/02, C12P17/12			
Applicant LONZA AG et al.			
<p>1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 5 sheets, including this cover sheet.</p> <p>3. This report is also accompanied by ANNEXES, comprising:</p> <p style="margin-left: 20px;">a. <input checked="" type="checkbox"/> sent to the applicant and to the International Bureau a total of 2 sheets, as follows:</p> <p style="margin-left: 40px;"><input checked="" type="checkbox"/> sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).</p> <p style="margin-left: 40px;"><input type="checkbox"/> sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.</p> <p style="margin-left: 20px;">b. <input type="checkbox"/> (sent to the International Bureau only) a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).</p>			
<p>4. This report contains indications relating to the following items:</p> <p><input checked="" type="checkbox"/> Box No. I Basis of the opinion</p> <p><input type="checkbox"/> Box No. II Priority</p> <p><input type="checkbox"/> Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p><input type="checkbox"/> Box No. IV Lack of unity of invention</p> <p><input checked="" type="checkbox"/> Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p><input type="checkbox"/> Box No. VI Certain documents cited</p> <p><input type="checkbox"/> Box No. VII Certain defects in the international application</p> <p><input type="checkbox"/> Box No. VIII Certain observations on the international application</p>			
Date of submission of the demand  24.05.2005		Date of completion of this report  30.01.2006	
Name and mailing address of the international preliminary examining authority:  European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016		Authorized Officer  Lejeune, R  Telephone No. +31 70 340-2347	



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ON PATENTABILITY**

International application No.  
PCT/EP2004/012065

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**Box No. I Basis of the report**

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1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.
- ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
- ☐ international search (under Rules 12.3 and 23.1(b))
  - ☐ publication of the international application (under Rule 12.4)
  - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)
2. With regard to the **elements\*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

**Description, Pages**

1-20 as originally filed

**Claims, Numbers**

1-13, 15-17 received on 24.05.2005 with letter of 24.05.2005

**Drawings, Sheets**

1/2, 2/2 as originally filed

- ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
- ☐ the description, pages
  - ☐ the claims, Nos.
  - ☐ the drawings, sheets/figs
  - ☐ the sequence listing (*specify*):
  - ☐ any table(s) related to sequence listing (*specify*):
4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
- ☐ the description, pages
  - ☐ the claims, Nos.
  - ☐ the drawings, sheets/figs
  - ☐ the sequence listing (*specify*):
  - ☐ any table(s) related to sequence listing (*specify*):

\* If item 4 applies, some or all of these sheets may be marked "superseded."

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**Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

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1. Statement

Novelty (N)	Yes: Claims	1-11,13,16,17
	No: Claims	12,15
Inventive step (IS)	Yes: Claims	
	No: Claims	1-13,15-17
Industrial applicability (IA)	Yes: Claims	1-13,15-17
	No: Claims	

2. Citations and explanations (Rule 70.7):

**see separate sheet**

**Re Item V.**

The following document is referred to in this communication:

- D1: SADA E ET AL: "PERFORMANCE OF AN ENZYME REACTOR UTILIZING A MAGNETIC FIELD" BIOTECHNOLOGY AND BIOENGINEERING, vol. 22, no. 1, 1980, pages 243-246
- D2: US-A-4 774 178 (EGERER ET AL) 27 September 1988 (1988-09-27)
- D3: WO 97/06248 A (ALLIED COLLOIDS LIMITED; ARMITAGE, YVONNE, CHRISTINE; HUGHES, JONATHAN) 20 February 1997 (1997-02-20)

**Novelty (Art 33(2) PCT)**

The subject matter of claims 1-11 is new because the prior art does not disclose a process for the preparation of polyacrylamide beads containing encapsulated cells where the suspension of cells with persulfate and acrylic monomers are mixed with an emulsion of an aqueous solution of a tertiary amine in a water-immiscible liquid.

The subject matter of claims 12 and 15 is not new because D3 discloses polyacrylamide beads containing encapsulated *Rhodococcus* cells containing a nitrilase, and the use of these beads for the transformation of a nitrile in an acid.

The subject matter of claim 17 is new.

**Inventive step (Art 33(3) PCT)**

The subject matter of claim 1 does not involve an inventive step because it is known from the prior art that the tertiary amine can be present in the water-immiscible liquid before the addition of the material to be immobilized with acrylic monomers and persulfate. For example D1 (see materials and methods section) discloses the preparation of polyacrylamide beads containing urease where the toluene-chloroform phase contains a surfactant and tetramethylethylenediamine. D2 discloses the preparation of

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(SEPARATE SHEET)**

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*Protaminobacter rubrum* containing beads where a suspension of polymerizable acrylic resin, cells and persulfate is added dropwise to a stirred silicone oil containing tetramethylethylenediamine.

Claims 2-13,15-17 do not appear to contain any additional features which, in combination with the features of any claim to which they refer, involve an inventive step as the relevant subject matter is either disclosed in the cited prior art or falls within the knowledge and ability of the skilled person.

**Further remarks**

The claim numbering is not correct: there is no claim 14.

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23.05.2005

Lonza AG

New claims

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1. A process for the preparation of polyacrylamide beads containing encapsulated cells comprising the steps of

- (i) providing an aqueous solution of a mixture of acrylic monomers,
- (ii) providing a suspension of cells in an aqueous solution of a persulfate
- 10 (iii) providing an emulsion of an aqueous solution of a tertiary amine in an water-immiscible liquid, which liquid optionally contains a surfactant,
- (iv) mixing the solution provided in step (i) and the suspension provided in step (ii)
- (v) adding the mixture obtained in step (iv) to the stirred emulsion provided in step (iii)
- 15 (vi) polymerizing the mixture of acrylic monomers and simultaneously encapsulating the cells to form polyacrylamide beads containing encapsulated cells,

wherein the polyacrylamide beads have a mechanical strength of at least 200 mN.

20 2. The process of claim 1 wherein the polyacrylamide beads have a size of 0.05 to 3 mm.

3. The process of claim 2 wherein the polyacrylamide beads have a size of 0.1 to 1.5 mm and a mechanical strength of at least 300 mN.

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4. The process of any of claims 1 to 3, wherein the ratio of dry cells/mixture of acrylic monomers is 0.001:1 to 1:1 (w/w).

5. The process of any of claims 1 to 4, wherein the ratio of dry cells/mixture of acrylic monomers is 0.2:1 to 0.9:1 (w/w).

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6. The process of any of claims 1 to 5 wherein the cell is a bacterial cell.

7. The process of claim 6 wherein the cell is a cell of a bacterium of the group nocardioform Actinomycetes or of the family Enterobacteriaceae.
8. The process of any of claims 1 to 7 wherein the tertiary amine is *N,N,N',N'*-tetra-  
5 methylethylenediamine or 3-(dimethylamino)propionitrile.
9. The process of any of claims 1 to 8 wherein the water-immiscible liquid is a mineral oil.
10. The process of any of claims 1 to 9 wherein no surfactant is used.
11. The process of any of claims 1 to 10 wherein the polyacrylamide beads formed in step (vi) are separated.
12. Polyacrylamide beads containing encapsulated cells obtainable by a process of any of  
15 claims 1 to 11 wherein the polyacrylamide beads have a mechanical strength of at least 200 mN.
13. The polyacrylamide beads of claim 12 wherein the encapsulated cells are cells of a  
20 strain of the genus *Rhodococcus* containing a nitrile hydratase.
15. The use of the polyacrylamide beads of claims 12 or 13 as a biocatalyst for the transformation of a substrate to a product.
16. The use of claim 15 wherein the substrate is a nitrile and the product is the  
25 corresponding amide.
17. The use of claim 16 wherein the nitrile is 3-cyanopyridine and the product is  
30 nicotinamide.